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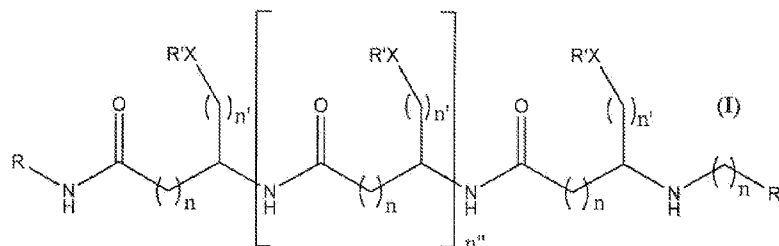
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(57) Abstract: A method of making a peptide of Formula (I) : is described, along with compounds and methods of sequencing. A method of making a peptide of Formula (I) is described, along with compounds and methods of sequencing. Further provided is a method for the synthesis of a solid-phase combinatorial library of compounds functionalized with natural and non natural groups displayed on a peptide scaffold, along with compounds useful for carrying out such methods.

SOLID PHASE SCAFFOLD BASED LIBRARIES

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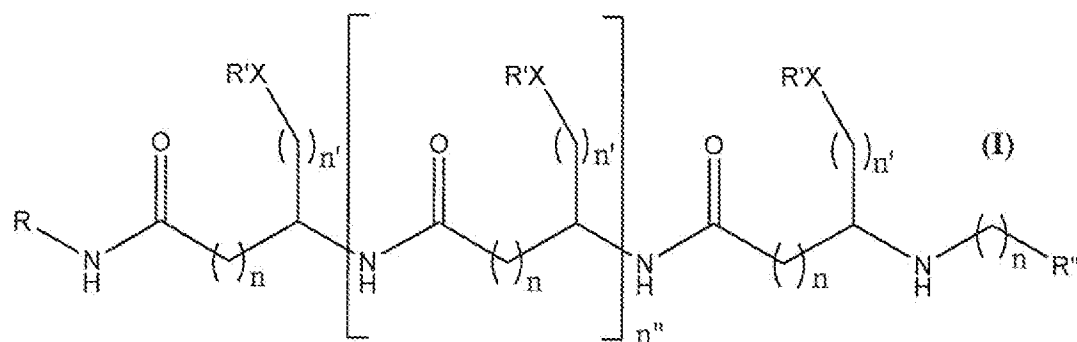
Background of the Invention

Classic solid-phase peptide libraries, while being a valuable tool for discovery of therapeutic agents and affinity binding ligands, suffer from one main limitation, that is, the difficulty and the high cost associated with the introduction of functional groups other than those carried by natural amino acids. In other words, exploring the chemical space outside that defined by the 20 natural amino acids is challenging under both technical and economic aspects. Chemical diversity, or chemical space, designates the range of functional groups and backbone properties comprised in the library. Besides natural amino acid residues, in fact, synthetic libraries can incorporate sugars, lipids, nucleobases, and non natural functionalities. The presence of these groups on the peptide backbone plays a crucial role in determining the biophysical and biochemical properties of the resulting molecule. In glycopeptides, for example, the introduction of sugar moieties on a peptide backbone determines the immunogenicity, pharmacokinetics, bioactivity and stability of the ligand. Lipoglycopeptides that display, besides amino acid functional groups, both sugar and fats frequently display antibiotics functions. Fully synthetic functional groups and reactive moieties can be introduced to impart other properties or allow further modifications.

Summary of the Invention

In order to benefit from the potential of a library with the widest possible chemical diversity and, at the same time, reduce the cost and the synthetic effort inherent to building such massive collection of compounds, we propose a method for the synthesis of a solid-phase combinatorial library of compounds functionalized with natural and non natural groups displayed on a peptide scaffold, along with compounds useful for carrying out such methods.

Thus a first aspect of the present invention is a method of making a peptide of
Formula I:



wherein:

R is a solid support;

each R' is an independently selected functional group;

R'' is a functional group;

each X is a linking group;

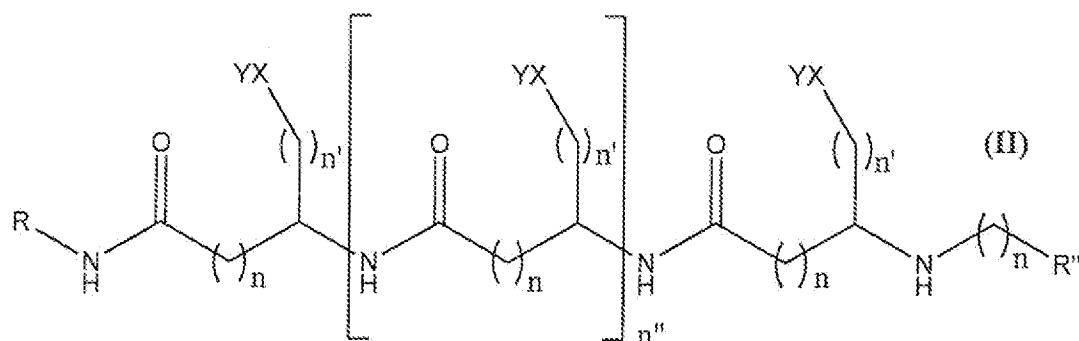
each n is 0, 1 or 2;

each n' is 0 or an integer of 1, 2, 3, 4 or 5; and

n'' is an integer of from 1, 2, 3 or 4 to 18 or 20;

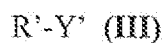
said method comprising the steps of:

(a) removing at least one protecting group from a compound of **Formula II**:



where R, R'', n, n' and n'' are as given above and each Y is an independently selected protecting group to produce a deprotected intermediate; then

(b) reacting said deprotected intermediate with compound of **Formula III**:



where R' is a functional group as given above and Y' is a reactive or functional group, to replace said at least one protecting group Y removed in step (a) with said functional group R'; and then

(c) iteratively repeating steps (a) and (b) at least once (*e.g.*, one, two, three or four times, up to eighteen or twenty times) until all of said protecting groups Y are replaced with said functional groups R'.

The present invention is explained in greater detail in the drawings herein and the specification below.

Detailed Description of the Preferred Embodiments

The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

The disclosures of all United States patents cited herein are to be incorporated herein by reference in their entirety.

A. Definitions.

"Peptides" of the invention, including both linear and cyclic, and combinations thereof, are compounds that contain at least one peptide bond. In general, such peptides comprise a polypeptide of 2, 3, 4 or 5 amino acids, up to 10, 12, 15 or 20 amino acids.

Amino acids used to carry out the present invention may be in D form, L form, or a combination thereof. (The single letter code for the standard amino acids is A (Ala), C (Cys), D (Asp), E (Glu), F (Phe), G (Gly), H (His), I (Ile), K (Lys), L (Leu), M (Met), N (Asn), P (Pro), Q (Gln), R (Arg), S (Ser), T (Thr), V (Val), W (Trp), and Y (Tyr)). Both natural and non-natural amino acids may be employed.

"Solid support" as used herein refers to an inert material or molecule to which a peptide ligand may be bound or coupled, either directly or indirectly through a linking group. The solid phase support is suitable for use in column chromatography or other types of purification. Solid supports include inorganic materials, organic materials, and combinations thereof. Examples of suitable solid support materials include membranes, semi-permeable membranes, capillaries, microarrays, multiple-well plates comprised of alumina, alumina supported polymers, polysaccharides including agarose, dextran, cellulose, chitosan, and polyacrylamide, polyacrylate, polystyrene, polyvinyl alcohol, glass, silica, silicon, zirconia, magnetite, semiconductors and combinations thereof. The solid support material may be in the form of beads, which are generally spherical. Alternatively, the support may be particulate or divided form having other regular or irregular shapes or it may be in the form of an integrated material such as a sheet, or a surface of a plate, tube, or well. Preferred solid support materials are those having minimal non-specific binding properties and that are physically and chemically resistant to the conditions used in peptide synthesis and coupling, such as organic solvents and acids, and in the purification process employed in this invention, such as changes in pH and ionic strength.

"Functional group" as used herein may be any suitable group or substituent, including but not limited to H, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloalkylalkenyl, cycloalkylalkynyl, heterocyclo, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, aryl, arylalkyl, arylalkenyl, arylalkynyl, heteroaryl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, alkoxy, halo, mercapto, azido, cyano, formyl, carboxylic acid, hydroxyl, nitro, acyl, aryloxy, alkylthio, amino, alkylamino, arylalkylamino, substituted amino, acylamino, acyloxy, ester, thioester, carboxylic thioester, ether, amide, amidino, sulfate, sulfoxyl, sulfonyl, sulfonamide, urea, alkoxyacylamino, aminoacyloxy, guanidino, aldehyde, keto, imine, nitrile, phosphate, thiol, epoxide, peroxide, thiocyanate, amidine, oxime, nitrile, diazo, etc., these terms including combinations of these groups (e.g. alkylated groups) as discussed further below.

"Alkyl" as used herein alone or as part of another group, refers to a straight or branched chain hydrocarbon containing from 1 to 10 or 20 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, n-decyl, and the like. "Lower alkyl" as used herein, is a subset of alkyl, in some embodiments preferred, and refers to a

straight or branched chain hydrocarbon group containing from 1 to 4 carbon atoms. Representative examples of lower alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, and the like. The term "alkyl" or "loweralkyl" is intended to include both substituted and unsubstituted alkyl or loweralkyl unless otherwise indicated and these groups may be substituted with groups selected from halo (e.g., haloalkyl), alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclo, heterocycloalkyl, hydroxyl, alkoxy (thereby creating a polyalkoxy such as polyethylene glycol), alkenyloxy, alkynyloxy, haloalkoxy, cycloalkoxy, cycloalkylalkyloxy, aryloxy, arylalkyloxy, heterocycloxy, heterocycloalkyloxy, mercapto, alkyl-S(O)_m, haloalkyl-S(O)_m, alkenyl-S(O)_m, alkynyl-S(O)_m, cycloalkyl-S(O)_m, cycloalkylalkyl-S(O)_m, aryl-S(O)_m, arylalkyl-S(O)_m, heterocyclo-S(O)_m, heterocycloalkyl-S(O)_m, amino, carboxy, alkylamino, alkenylamino, alkynylamino, haloalkylamino, cycloalkylamino, cycloalkylalkylamino, arylamino, arylalkylamino, heterocycloamino, heterocycloalkylamino, disubstituted-amino, acylamino, acyloxy, ester, amide, sulfonamide, urea, alkoxyacylamino, aminoacyloxy, nitro or cyano where m= 0, 1, 2 or 3.

"Alkenyl" as used herein alone or as part of another group, refers to a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms (or in loweralkenyl 1 to 4 carbon atoms) which include 1 to 4 double bonds in the normal chain. Representative examples of alkenyl include, but are not limited to, vinyl, 2-propenyl, 3-butenyl, 2-butenyl, 4-pentenyl, 3-pentenyl, 2-hexenyl, 3-hexenyl, 2,4-heptadiene, and the like. The term "alkenyl" or "loweralkenyl" is intended to include both substituted and unsubstituted alkenyl or loweralkenyl unless otherwise indicated and these groups may be substituted with groups as described in connection with alkyl and loweralkyl above.

"Alkynyl" as used herein alone or as part of another group, refers to a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms (or in loweralkynyl 1 to 4 carbon atoms) which include 1 triple bond in the normal chain. Representative examples of alkynyl include, but are not limited to, 2-propynyl, 3-butylnyl, 2-butylnyl, 4-pentylnyl, 3-pentylnyl, and the like. The term "alkynyl" or "loweralkynyl" is intended to include both substituted and unsubstituted alkynyl or loweralkynyl unless otherwise indicated, and these groups may be substituted with the same groups as set forth in connection with alkyl and loweralkyl above.

"Cycloalkyl" as used herein alone or as part of another group, refers to a saturated or partially unsaturated cyclic hydrocarbon group containing from 3, 4 or 5 to 6, 7 or 8 carbons

(which carbons may be replaced in a heterocyclic group as discussed below). Representative examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. These rings may be optionally substituted with additional substituents as described herein such as halo or loweralkyl. These rings include those that form chelates with metals or are chelated with metals. The term "cycloalkyl" is generic and intended to include heterocyclic groups as discussed below unless specified otherwise.

"Heterocyclic group" or "heterocyclo" as used herein alone or as part of another group, refers to an aliphatic (e.g., fully or partially saturated heterocyclo) or aromatic (e.g., heteroaryl) monocyclic- or a polycyclic-ring system, including those forming chelates with metals. Monocyclic ring systems are exemplified by any 5 or 6 membered ring containing 1, 2, 3, or 4 heteroatoms independently selected from oxygen, nitrogen and sulfur. The 5 membered ring has from 0-2 double bonds and the 6 membered ring has from 0-3 double bonds. Representative examples of monocyclic ring systems include, but are not limited to, azetidine, azepine, aziridine, diazepine, 1,3-dioxolane, dioxane, dithiane, furan, imidazole, imidazoline, imidazolidine, isothiazole, isothiazoline, isothiazolidine, isoxazole, isoxazoline, isoxazolidine, morpholine, oxadiazole, oxadiazoline, oxadiazolidine, oxazole, oxazoline, oxazolidine, piperazine, piperidine, pyran, pyrazine, pyrazole, pyrazoline, pyrazolidine, pyridine, pyrimidine, pyridazine, pyrrole, pyrroline, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, tetrazine, tetrazole, thiadiazole, thiadiazoline, thiadiazolidine, thiazole, thiazoline, thiazolidine, thiophene, thiomorpholine, thiomorpholine sulfone, thiopyran, triazine, triazole, trithiane, and the like. Bicyclic ring systems are exemplified by any of the above monocyclic ring systems fused to an aryl group as defined herein, a cycloalkyl group as defined herein, or another monocyclic ring system as defined herein. Representative examples of bicyclic ring systems include but are not limited to, for example, benzimidazole, benzothiazole, benzothiadiazaole, benzothiophene, benzoxadiazole, benzoxazole, benzofuran, benzopyran, benzothiopyran, benzodioxine, 1,3-benzodioxole, cinnoline, indazole, indole, indoline, indolizine, naphthyridine, isobenzofuran, isobenzothiophene, isoindole, isoindoline, isoquinoline, phthalazine, purine, pyranopyridine, quinoline, quinolizine, quinoxaline, quinazoline, tetrahydroisoquinoline, tetrahydroquinoline, thiopyranopyridine, and the like. These rings include quaternized derivatives thereof and may be optionally substituted with additional functional groups, including but not limited to halo, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclo, heterocycloalkyl, hydroxyl, alkoxy, alkenyloxy, alkynyloxy, haloalkoxy, cycloalkoxy, cycloalkylalkyloxy, aryloxy,

arylalkyloxy, heterocyclooxy, heterocyclolalkyloxy, mercapto, alkyl-S(O)_m, haloalkyl-S(O)_m, alkenyl-S(O)_m, alkynyl-S(O)_m, cycloalkyl-S(O)_m, cycloalkylalkyl-S(O)_m, aryl-S(O)_m, arylalkyl-S(O)_m, heterocyclo-S(O)_m, heterocycloalkyl-S(O)_m, amino, alkylamino, alkenylamino, alkynylamino, haloalkylamino, cycloalkylamino, cycloalkylalkylamino, arylamino, arylalkylamino, heterocycloamino, heterocycloalkylamino, disubstituted-amino, acylamino, acyloxy, ester, amide, sulfonamide, urea, alkoxyacylamino, aminoacyloxy, nitro or cyano where m = 0, 1, 2 or 3.

"Aryl" as used herein alone or as part of another group, refers to a monocyclic carbocyclic ring system or a polycyclic carbocyclic fused ring system having one or more aromatic rings. Representative examples of aryl include, azulenyl, indanyl, indenyl, naphthyl, phenyl, tetrahydronaphthyl, and the like. The term "aryl" is intended to include both substituted and unsubstituted aryl unless otherwise indicated and these groups may be substituted with the same groups as set forth in connection with alkyl and loweralkyl above.

"Arylalkyl" as used herein alone or as part of another group, refers to an aryl group, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of arylalkyl include, but are not limited to, benzyl, 2-phenylethyl, 3-phenylpropyl, 2-naphth-2-ylethyl, and the like.

"Heteroaryl" as used herein is as described in connection with heterocyclo above.

"Alkoxy" as used herein alone or as part of another group, refers to an alkyl or loweralkyl group, as defined herein (and thus including substituted versions such as polyalkoxy), appended to the parent molecular moiety through an oxy group, -O-. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, 2-propoxy, butoxy, tert-butoxy, pentyloxy, hexyloxy and the like.

"Halo" as used herein refers to any suitable halogen, including -F, -Cl, -Br, and -I.

"Mercapto" as used herein refers to a -SH group.

"Azido" as used herein refers to an -N₃ group.

"Cyano" as used herein refers to a -CN group.

"Formyl" as used herein refers to a -C(O)H group.

"Carboxylic acid" as used herein refers to a -C(O)OH group.

"Hydroxyl" as used herein refers to an -OH group.

"Nitro" as used herein refers to an -NO₂ group.

"Acyl" as used herein alone or as part of another group refers to a $-C(O)R$ radical, where R is any suitable substituent such as aryl, alkyl, alkenyl, alkynyl, cycloalkyl or other suitable substituent as described herein.

"Alkylthio" as used herein alone or as part of another group, refers to an alkyl group, as defined herein, appended to the parent molecular moiety through a thio moiety, as defined herein. Representative examples of alkylthio include, but are not limited, methylthio, ethylthio, tert-butylthio, hexylthio, and the like.

"Amino" as used herein means the radical $-NH_2$.

"Alkylamino" as used herein alone or as part of another group means the radical $-NHR$, where R is an alkyl group.

"Arylalkylamino" as used herein alone or as part of another group means the radical $-NHR$, where R is an arylalkyl group.

"Disubstituted-amino" as used herein alone or as part of another group means the radical $-NR_aR_b$, where R_a and R_b are independently selected from the groups alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclo, heterocycloalkyl.

"Acylamino" as used herein alone or as part of another group means the radical $-NR_aR_b$, where R_a is an acyl group as defined herein and R_b is selected from the groups hydrogen, alkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclo, heterocycloalkyl.

"Acyloxy" as used herein alone or as part of another group means the radical $-OR$, where R is an acyl group as defined herein.

"Ester" as used herein alone or as part of another group refers to a $-C(O)OR$ radical, where R is any suitable substituent such as alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

"Amide" as used herein alone or as part of another group refers to a $-C(O)NR_aR_b$ radical, where R_a and R_b are any suitable substituent such as alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

"Sulfoxyl" as used herein refers to a compound of the formula $-S(O)R$, where R is any suitable substituent such as alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

"Sulfonyl" as used herein refers to a compound of the formula $-S(O)(O)R$, where R is any suitable substituent such as alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

"Sulfonate" as used herein refers to a compound of the formula $-S(O)(O)OR$, where R is any suitable substituent such as alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

"Sulfonic acid" as used herein refers to a compound of the formula $-S(O)(O)OH$.

"Sulfonamide" as used herein alone or as part of another group refers to a $-S(O)_2NR_aR_b$ radical, where R_a and R_b are any suitable substituent such as H, alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

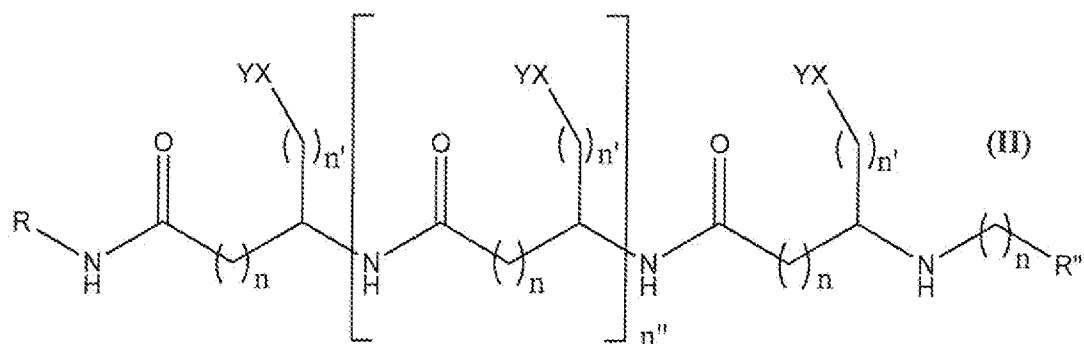
"Urea" as used herein alone or as part of another group refers to an $-N(R_c)C(O)NR_aR_b$ radical, where R_a , R_b and R_c are any suitable substituent such as H, alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

"Alkoxyacylamino" as used herein alone or as part of another group refers to an $-N(R_a)C(O)OR_b$ radical, where R_a , R_b are any suitable substituent such as H, alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

"Aminoacyloxy" as used herein alone or as part of another group refers to an $-OC(O)NR_aR_b$ radical, where R_a and R_b are any suitable substituent such as H, alkyl, cycloalkyl, alkenyl, alkynyl or aryl.

1. Compounds.

As noted above, compounds useful in the present invention are generally compounds of **Formula II**:



wherein:

R is a solid support;

each R' is an independently selected functional group;

R'' is a functional group;

each n is 0, 1 or 2;

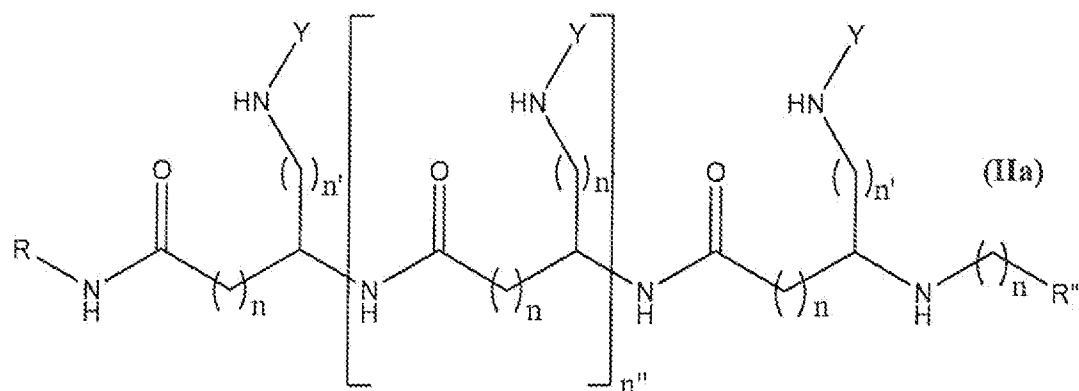
each n' is 0 or an integer of 1, 2, 3, 4 or 5; and

n'' is an integer of from 1, 2, 3, 4, 5 or 6, up to 18 or 20;

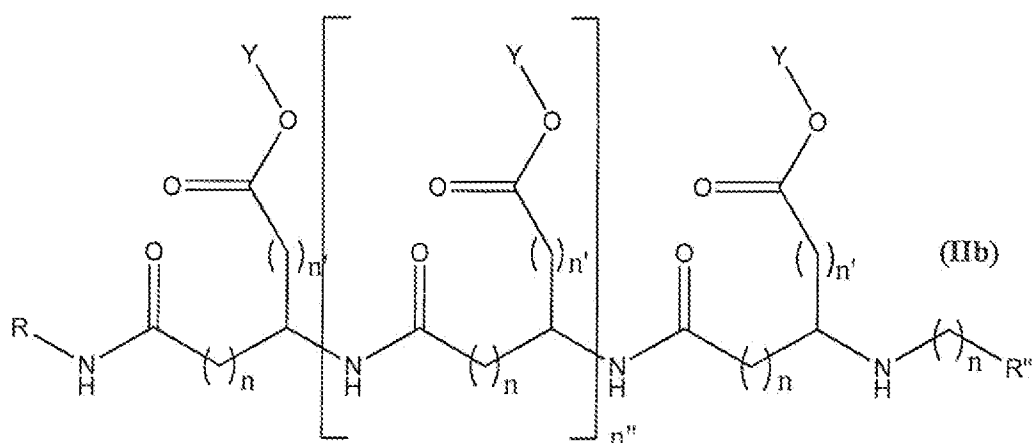
each X is a linking group (*e.g.*, secondary or tertiary amine, ester, amide); and

each Y is an independently selected protecting group.

In some embodiments the compound is a compound of of Formula IIa:



In some embodiments, the compound is a compound of **Formula IIb**:



In some embodiments, at least three, four, five, or six (up to eighteen or twenty) of said protecting groups Y are different from one another.

Numerous suitable protecting groups are known. See, e.g., A. Isidro-Llobet, M. Alvarez, and F. Albericio, Amino Acid-Protecting Groups, *Chem. Rev.* **109**, 2455–2504 (2009). Non-limiting examples of protecting groups include, but are not limited to:

Alkaline-stable amino protecting groups such as: 2-(2-Nitrophenyl)propyloxycarbonyl (NPPOC), 2-(3,4-Methylenedioxy-6-nitrophenyl)propyloxycarbonyl (MNPPOC), 2-(4-Biphenyl)isopropoxycarbonyl (Bpoc), 2,2,2-Trichloroethyloxycarbonyl (Troc), 2,4-Dinitrobenzenesulfonyl (dNBS), 2-Chlorobenzylloxycarbonyl (Cl-Z), 2-Nitrophenylsulfenyl (Nps), 4-Methyltrityl (Mtt), 9-(4-Bromophenyl)-9-fluorenyl (BrPhF), Allyloxycarbonyl (Alloc), Azidomethoxycarbonyl

(Azoc), Benzyloxycarbonyl (Z), o-Nitrobenzyloxycarbonyl (oNZ) and 6-Nitroveratryloxycarbonyl (NVOC), p-Nitrobenzyloxycarbonyl (pNZ), Propargyloxycarbonyl (Poc), tert-Butyloxycarbonyl (Boc), Trityl (Trt), α,α -Dimethyl-3,5-dimethoxybenzyloxycarbonyl (Ddz), and α -Azido Carboxylic Acids, etc.

Alkaline-labile amino protecting groups, such as: (1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-ethyl) (Dde), (1,1-Dioxobenzo[b]thiophene-2-yl)methyloxycarbonyl (Bsmoc), (1,1-Dioxonaphtho[1,2-b]thiophene-2-yl)methyloxycarbonyl (r-Nsmoc), 1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde), 2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc), 2-(4-Sulfophenylsulfonyl)ethoxycarbonyl (Sps), 2,7-Di-tert-butyl-Fmoc (Fmoc*), 2-[Phenyl(methyl)sulfonio]ethyloxycarbonyl tetrafluoroborate (Pms), 2-Fluoro-Fmoc (Fmoc(2F)), 2-Monoisooctyl-Fmoc (mio-Fmoc) and 2,7-Diisooctyl-Fmoc (dio-Fmoc), 9-Fluorenylmethoxycarbonyl (Fmoc), Ethanesulfonyl ethoxycarbonyl (Esc), and Tetrachlorophthaloyl (TCP), etc.

Alkaline-stable carboxylic acid protecting groups, such as: (2-Phenyl-2-trimethylsilyl)ethyl (PTMSE), 1,1-Dimethylallyl (Dma), 2-(Trimethylsilyl)isopropyl (Tmsi), 2,2,2-Trichloroethyl (Tce), 2,4-Dimethoxybenzyl (Dmb), 2-Chlorotriptyl (2-Cl-Trt), 2-Phenylisopropyl (2-PhiPr), 2-Phenylisopropyl (2-PhiPr), 2-Trimethylsilyl ethyl (TMSE), 4-(3,6,9-Trioxadecyl)oxybenzyl (TEGBz or TEGBN), 4,5-Dimethoxy-2-nitrobenzyl (Dmnb), 5-Phenyl-3,4-ethylenedioxythienyl Derivatives (Phenyl-EDOTn), Allyl (Al), Benzyl (Bn), Cyclohexyl (cHx), Pentaamine Cobalt(III), Phenacyl (Pac), p-Hydroxyphenacyl (pHP), p-Nitrobenzyl (pNB), tert-Butyl (tBu), β -3-Methylpent-3-yl (Mpe), and β -Menthyl (Men), etc.

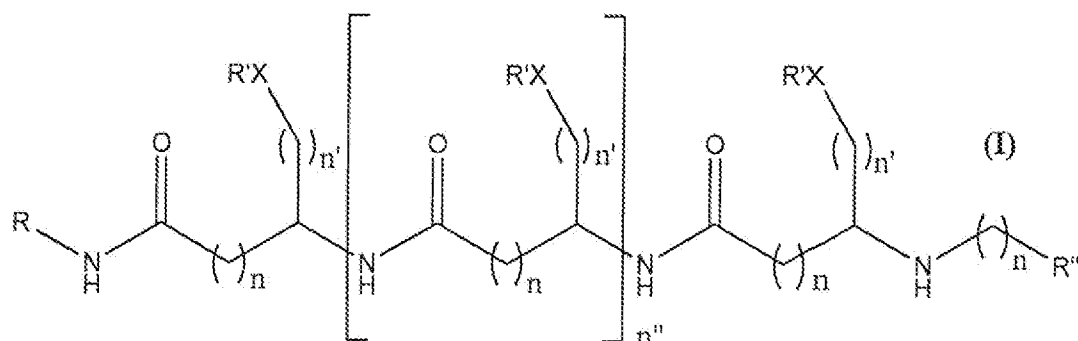
Alkaline-labile carboxylic acid protecting groups: 9-Fluorenylmethyl (Fm), Methyl (Me) and Ethyl (Et), Carbamoylmethyl (Cam), and 4-(N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino)benzyl (Dmab), etc.

Additional examples include but are not limited to those set forth in US Patents Nos. 8,299,279; 8,129,561; 8,008,500; 7,713,927; and 7,521,529, the disclosures of all of which are incorporated herein by reference.

Compounds of Formulas II, IIa and IIb are made in accordance with known techniques or as described below, or modifications thereof that will be apparent to those skilled in the art.

2. Methods of use.

As noted above, the present invention provides a method of making a peptide of **Formula I**:



wherein:

R is a solid support;

each R' is an independently selected functional group;

R'' is a functional group;

X is a linking group;

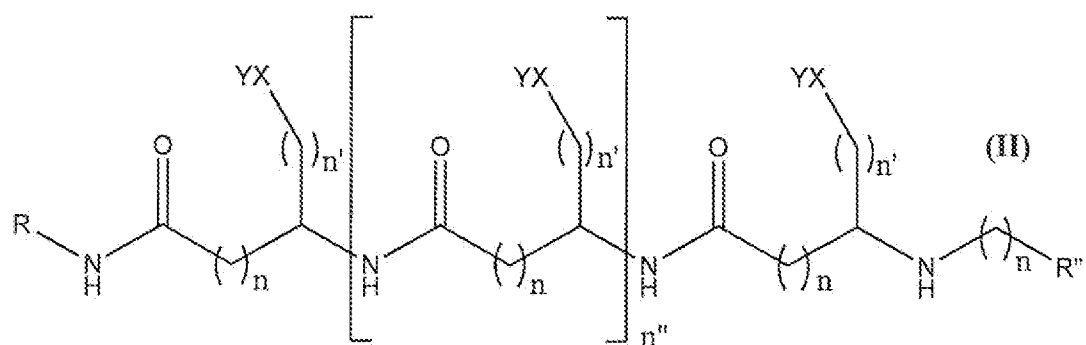
each n is 0, 1 or 2;

each n' is 0 or an integer of from 1 to 5; and

n'' is an integer of from 1 to 20;

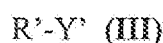
said method comprising the steps of:

(a) removing at least one protecting group from a compound of **Formula II**:



where R, R'', X, n, n' and n'' are as given above and each Y is an independently selected protecting group to produce a deprotected intermediate; then

(b) reacting said deprotected intermediate with a compound of **Formula III**:



where R' is a functional group as given above and Y' is a reactive or functional group, to replace said at least one protecting group Y removed in step (a) with said functional group R'; and then

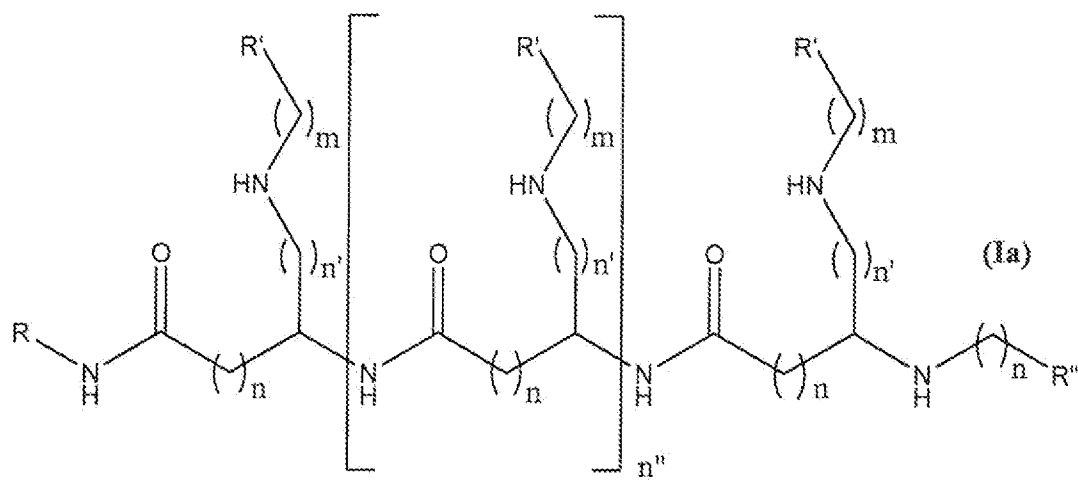
(c) iteratively repeating steps (a) and (b) at least once (*e.g.*, two, three or four times, up to 20 or 22 times) until all of said protecting groups Y are replaced with said functional groups R'.

In some embodiments, at least three, four or five (up to 20 or 22) of said functional groups R' are different from one another; and at least three, four or five (up to 20 or 22) of said protecting groups Y are different from one another.

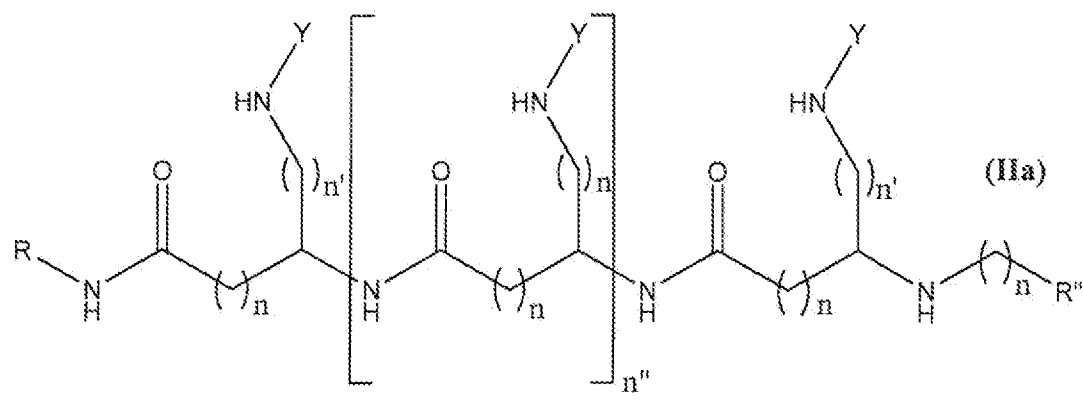
While like protecting groups Y removed in each step (b) can be replaced by like (that is, the same) functional groups R' in each immediate subsequent step (c), they can also be replaced by different functional groups R' to generate a peptide library, as described below).

The step of removing or selectively removing protecting groups can be carried out in accordance with known techniques or variations thereof that will be apparent to those skilled in the art. *See, e.g.*, US Patent No. 7,521,529; A. Isidro-Llobet, M. Alvarez, and F. Albericio, Amino Acid-Protecting Groups, *Chem. Rev.* **109**, 2455–2504 (2009). Orthogonal deprotection (that is, the selective deprotection of different protecting groups or a subset of protecting groups during each cyclically repeated deprotection step) can be achieved in accordance with known techniques, such as: modulating deprotection conditions (for example, by increasing the acid content in subsequent deprotecting steps to remove increasingly more resistant acid-labile protecting groups, changing deprotection conditions (*e.g.*, from a reduced pH conditions, to hydrazine conditions for hydrazine-labile protecting groups), etc., including combinations thereof.

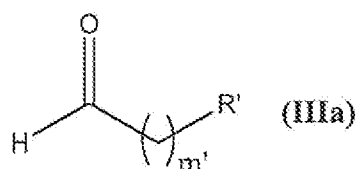
The step of reacting the deprotected intermediate with a compound of Formula III can likewise be carried out in accordance with known techniques, the specific choice of which will depend upon the specific linking group employed. For example, where the compound of **Formula I** is a compound of **Formula Ia**:



(where each m is independently 1, 2 or 3), and the compound of **Formula II** is a compound of **Formula IIa**:

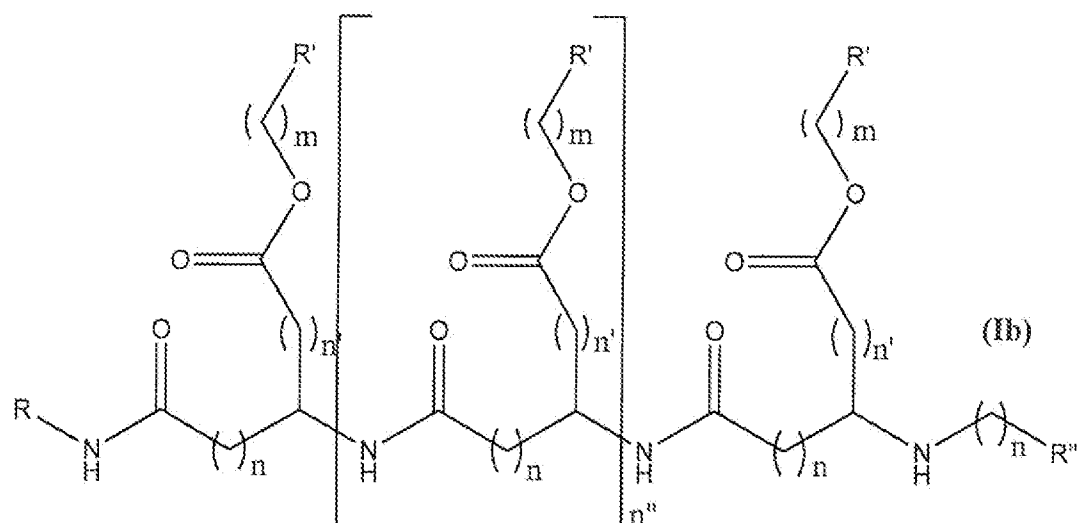


Then the compound of **Formula III** can be a compound of **Formula IIIa**:

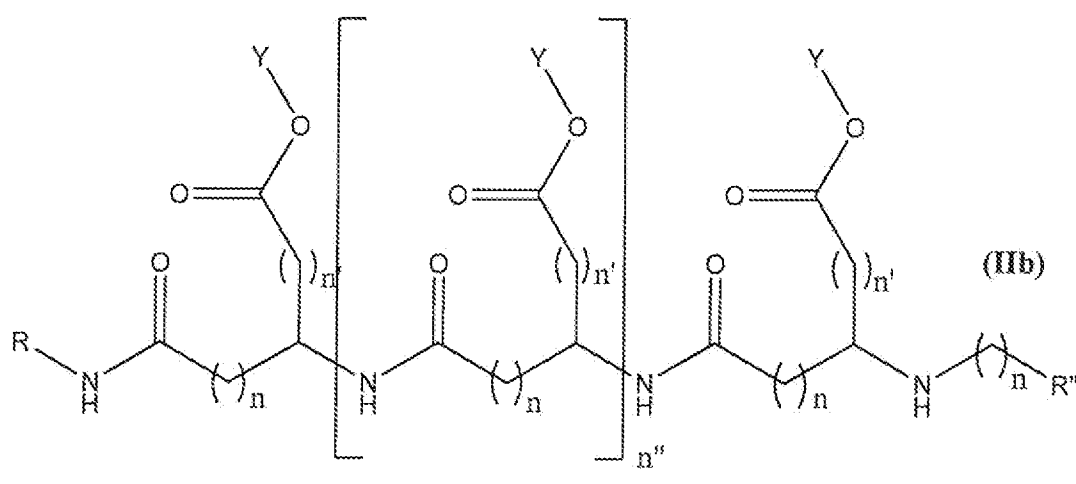


where m' is 0, 1 or 2. In this embodiment, the reacting step (b) comprises a reductive amination reaction. The conditions for reductive amination are not critical and this reaction can be carried out in accordance with known techniques. *See, e.g.*, US Patents Nos. 8,287,850; 7,821,006; 5,750,790; 5,387,696; etc. In general the reaction may be carried out in a polar organic solvent such as an alcohol (*e.g.*, methanol, ethanol, isopropanol, etc.) with catalyst such as a hydride catalyst, typically for a time of 1 to 2 hours or more.

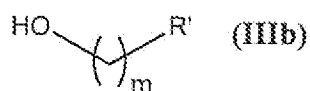
In the alternative, when the compound of **Formula I** is a compound of **Formula Ib**:



where each m is independently 1, 2 or 3; and the compound of **Formula II** is a compound of **Formula IIb**:



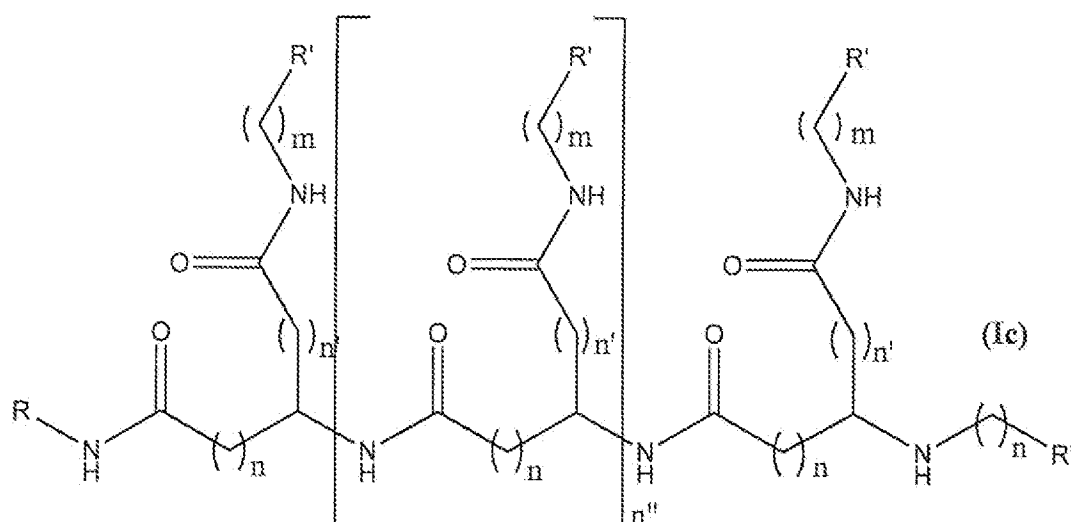
Then the compound of **Formula III** can be a compound of **Formula IIIb**:



where m is as given above, and the reacting step (b) may comprise an esterification reaction. The conditions for the esterification reaction are likewise not critical and this reaction can be carried out in accordance with known techniques. *See, e.g.*, US Patent Nos. 7,943,792; 7,857,944; 7,767,769; 4,610,825; 4,457,868; etc. In some embodiments, the reaction may be carried out by first activating the carboxyl group with a carbodiimide, cyanuric chloride, phosphonium, aminium or uronium reagents, then reacting the activated carboxyl group with

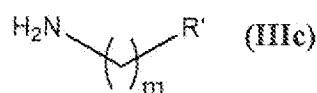
the compound of Formula IIIb in an organic polar solvent, such as dimethyl formamide, dichloromethane, dimethylsulfoxide, or water, etc.

In still another embodiment, where the compound of Formula I is a compound of Formula Ic:



where each m is independently 1, 2 or 3;

then the compound of Formula II is a compound of Formula IIb as described above, but the compound of Formula III is a compound of Formula IIIc:



where m is as given above, and the reacting step (b) is carried out in like manner as an amidation reaction.

All reacting steps can be conveniently carried out on a solid phase with washing steps interposed as desired in accordance with known techniques, with the compounds of Formulas I, Ia and Ib finally released or cleaved from the solid support ®- in accordance with known techniques depending upon the particular solid support employed.

In some embodiments, the compound of Formula III, IIIa, or IIIb in each reacting step comprises a plurality of 2, 3, 4 or 5 or more (up to 10, 20, or 30 or more) of different compounds of Formula III, IIIa or IIIb, each with a different R' group (and optionally but preferably the same Y' group), all concurrently reacted in the same step, to facilitate the generation of a library of compounds I, Ia, or Ib. During subsequent iteratively repetitions of steps (b) and (c), different compounds of Formula III, IIIa, or IIIb may be used, with different

groups Y' from that used in previous steps (to achieve the desired orthogonal reaction conditions), and the same or different groups R' from previous steps).

3. Utility.

Peptidomimetics and peptidomimetic libraries produced by the methods of the present invention can be used in accordance with known techniques. The methods can be used to produce a single predetermined peptide of known activity and predetermined utility. In the case of peptide libraries, the peptide library products may be used in known techniques including but not limited to drug discovery (that is, the discovery of active agents or compounds from the library that specifically bind to, agonize, and/or antagonize a drug target such as a G-protein coupled receptor, cell membrane pore or ion gate, circulating hormone, etc.), epitope mapping, the development or identification of affinity ligands, etc.

The present invention is explained in greater detail in the following non-limiting Examples.

EXAMPLES

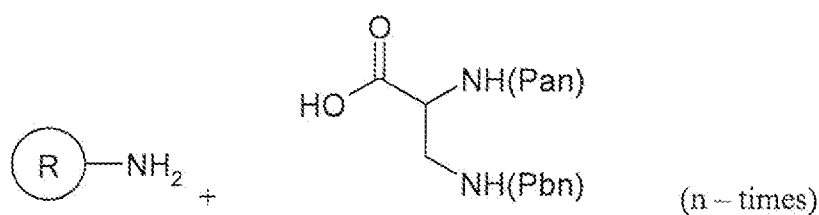
This disclosure presents a method for the **synthesis of solid-phase highly structurally and chemically diverse combinatorial libraries of peptide-based compounds functionalized with natural and non-natural moieties**. The base peptide structure consists of a protected poly-diaminoacid, e.g. poly-DAP (diaminopropionic acid), poly-DAB (diaminobutyric acid), poly-DAP (poly-diaminopentanoic acid, else poly-ornithine), poly-lysine, etc., or a poly-carboxylic, e.g. poly-aspartic or poly-glutamic acid, backbone / scaffold with linear or cyclic structure, or a combination thereof. The functional groups appended on such peptide scaffold can be either natural biomonomers, such as the side chain of natural amino acids, sugars, lipids or nucleobases, or non natural moieties. In case of the polyamine scaffold, these functional groups are provided with an aldehyde group, that is, they are in the form of a functional aldehyde R-COH, wherein R is one of the above listed functional moieties. The functional aldehyde is coupled to the ω -amino group of each diaminoacid on the peptide scaffold by reductive amination. In case of the polycarboxylic scaffold, the functional groups are provided with a hydroxyl form, that is, they are in the form of a functional alcohol R-OH, wherein R is one of the above listed functional moieties. The

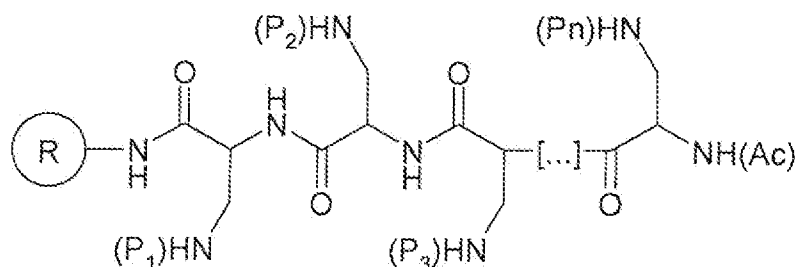
alcohol is coupled to the ω -carboxyl group of each carboxyl amino acid on the peptide scaffold by formation of ester bond.

Both methods (with poly-diamino acid or poly-carboxyl amino acid) comprises $1 + 2n$ steps, wherein n is the number of amino acid residues comprised in the scaffold:

1. *Synthesis of a protected poly-diamino acid or poly-carboxyl amino acid scaffold*, or any analogous structure. Analogous diamino acids are 2,2-diaminoethanoic acid, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, 2,5-diaminopentanoic acid (ornithine), 2,6-diaminohexanoic acid (lysine), 2,7-diaminoheptanoic acid, and the like. Analogous carboxyl amino acids are glutamic acid, aspartic acid, and the like. The groups protecting each ω -amino group and ω -carboxyl group on the scaffold of the poly-diamino acid and the poly-carboxyl amino acid respectively are orthogonal to each other, that is, the conditions required to cleave each protecting group are different from the conditions required to cleave any other protecting group.
2. *Removal of the first protecting group* with the appropriate cleavage conditions.
3. *First reductive amination* to couple the desired functional aldehyde on a deprotected primary amino group / *First esterification* to couple the desired alcohol on a deprotected carboxyl group.
4. *Removal of the second protecting group and second reductive amination / second esterification* to couple the desired functional aldehyde / alcohol respectively.
5. *Iteration of step 4 on the remaining residues.*

In the first example reported herein the protected amino acid for the synthesis of the scaffold is 2,3-diaminopropionic acid.



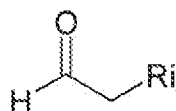


Wherein $P_{\alpha N}$ is the protecting group on the α -amino group of the amino acid, while $P_{\beta N}$ is the protecting group on the β -amino group, that is, the amino group on the side chain of the amino acid. A suitable example of $P_{\alpha N}$ is fluorenylmethoxycarbonyl (Fmoc). Suitable examples of $P_{\beta N}$ are:

- 1 – Alloc: removed in minutes by 0.1 eq of palladium(0) tetrakis(triphenylphosphine) with 10 eq. of phenylsilane as scavenger in DCM.
- 2 – tBoc: removed in 50% TFA in DCM
- 3 – Nde, Dde and ivDde: cleaved by 2% hydrazine in DMF
- 4 – Fmoc: removed by 20% piperidine in DMF
- 5 – Mmt and Mtt: removed by 1% TFA in DCM
- 6 – Z: boron trifluoride in methanol

These groups are also mutually orthogonal.

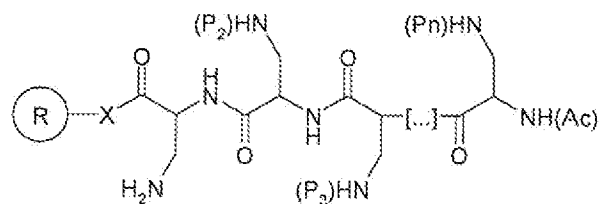
The functional aldehyde has the general structure



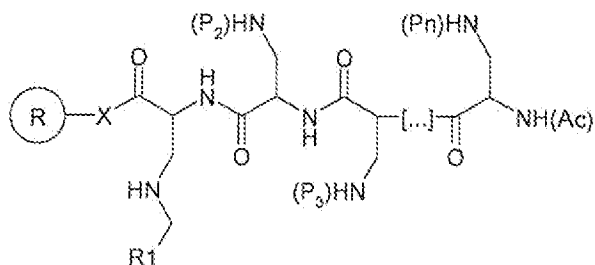
wherein R_i is the desired functional group: 1) the side chain of natural and non natural amino acid, 2) a sugar, 3) a lipid, 4) a nucleobase, 5) a synthetic species.

The synthesis proceeds as a sequence of selective deprotection steps followed by reductive amination.

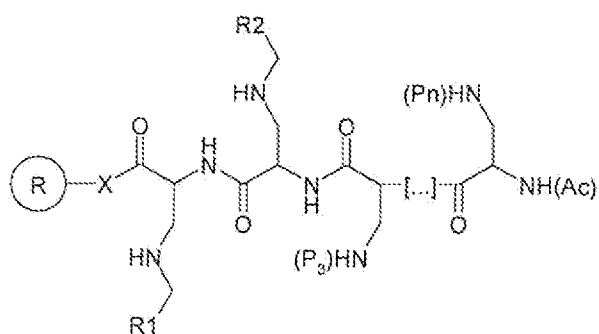
- 1 – Deprotection of the first amino group



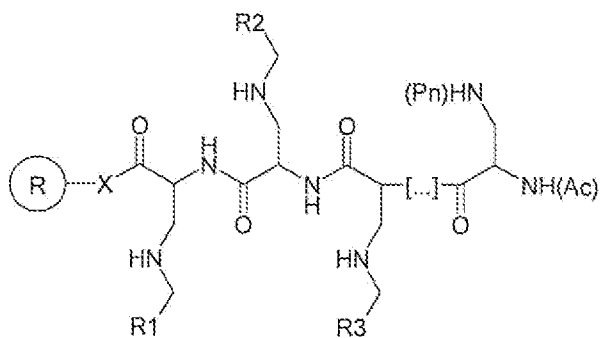
2 – First reductive alkylation



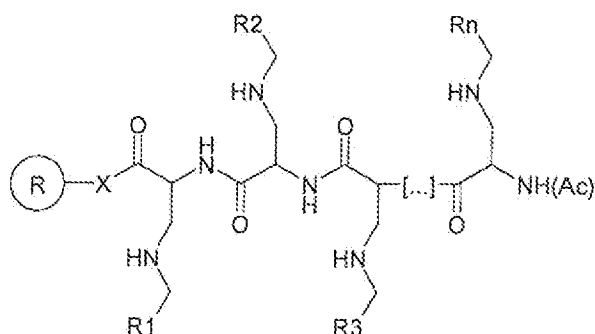
3 – Deprotection of the second amino group and second reductive alkylation



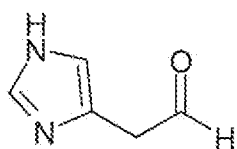
4 – Deprotection of the third amino group and third reductive alkylation



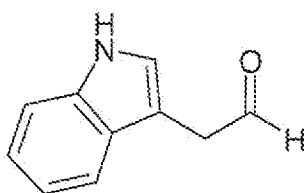
n – Deprotection of the n-th amino group and n-th reductive alkylation



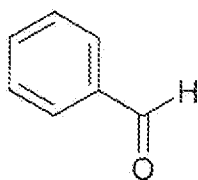
Two examples of scaffolds: analogues of HWK and HFK (IgG-binding ligands)



Imidazolecarboxaldehyde (side group of Histidine)

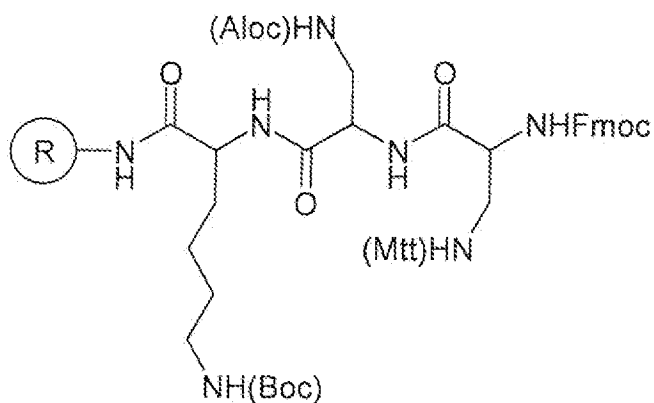


Indolecarboxaldehyde (side group of Tryptophan)



Benzaldehyde (side group of Phenylalanine)

Protected scaffold: Fmoc-Dap(Mtt)-Dap(Aloc)-Lys(Boc)-A-Toyopearl resin



Deprotection conditions:

Mtt: 5% TFA (+ 5% TIPS) in DCM (3 x 10min)

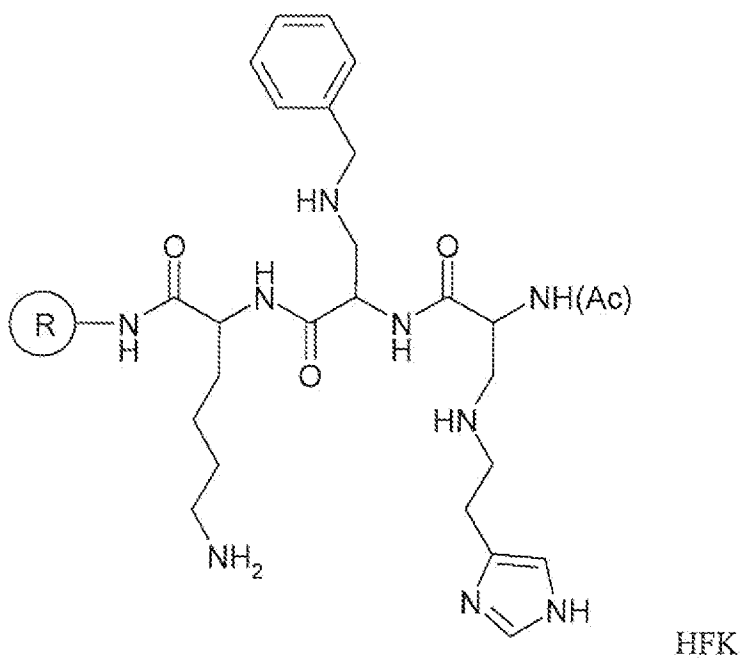
Boc: 50% TFA in DCM (2 x 30min)

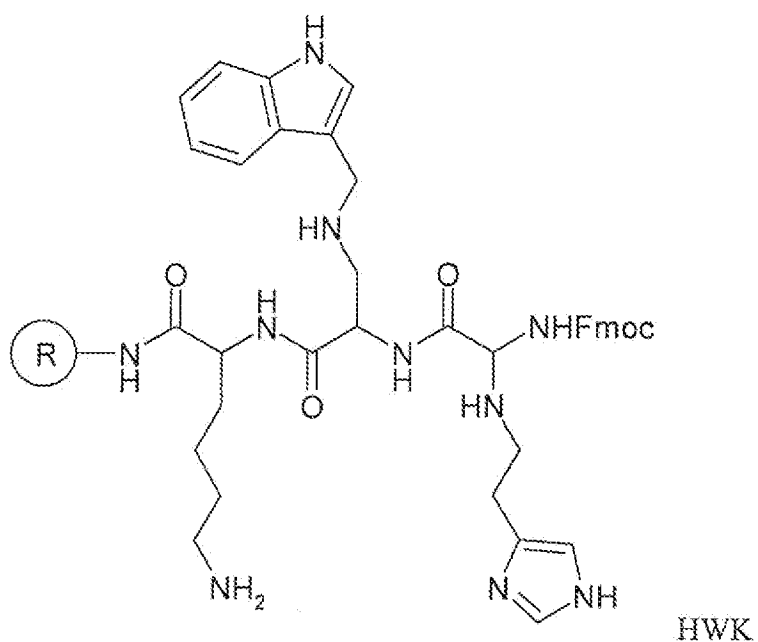
Aloc: $\text{Pd}(\text{Ph}_3\text{P})_4 + \text{PhSiH}_3$ (2 x 5 min)

Conditions of reductive amination:

1eq. of Sodium Triacetoxyborohydride

1eq. of functional aldehyde





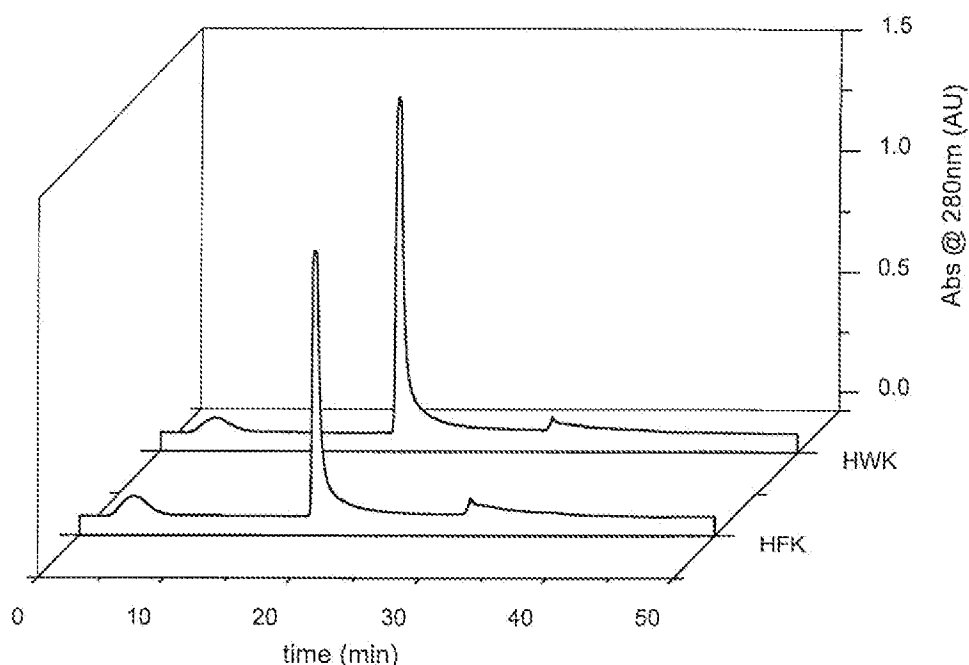
Chromatographic validation (IgG injection)

Buffer A: PBS pH 7.4

Buffer B: 0.2M Acetate pH 4.0

Buffer C: 0.85% Phosphoric acid

Time (min)	Flow (ml/min)	A %	B %	C %
0	0.05	100	0	0
10	0.2	100	0	0
15	0.4	0	100	0
25	0.3	0	0	100
35	0.4	100	0	0
50	0	100	0	0



SEQUENCING PROCESSES

Given a solid-phase combinatorial library of compounds of **Formula Ib** as described above, it will be noted that all the functional groups in the library are covalently attached to the backbone by ester bond. Using ester bond is crucial for the post-screening hit identification.

During library screening, beads carrying ligands that bind to the target molecule are selected. After the screening, these ligands are to be sequenced to determine which functional groups are present on the ligand and in which position on the scaffold each group is located. Sequence determination is usually a rather complex and/or costly procedure for peptides and related compounds. Edman degradation, for example, costs around \$ 250 per bead and it is not amenable to the sequencing of head-to-tail cyclic peptides. Mass spectrometry, while less expensive, is rather complex for sequencing cyclic peptides and requires a considerable guesswork.

The functional group being appended on the backbone via ester bond considerably facilitates the sequencing of the selected beads. In fact, by individually treating each bead in alkaline conditions, all the ester bonds are rapidly hydrolyzed and the functional groups are released in solution. The functional groups present in solution can then be determined by measuring their mass via mass spectrometry. It is paramount that the selected functional

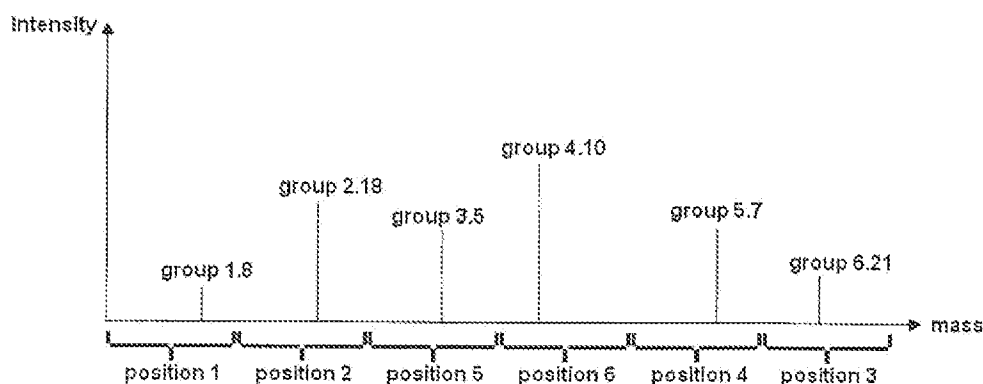
groups have different molecular weight, so that their unequivocal identification be solely based on the measurement of their molecular weight.

A library of n-mers can be construed using m functional hydroxyls of **Formula IIIb** above. By treating each bead in alkaline conditions, n functional hydroxyls are released from the bead to the liquid phase. A single mass spectrum is sufficient to determine which groups were appended on the peptide backbone. In order to determine the position, it is necessary to refer to the initial library design as follows.

The group of m functional hydroxyls selected for constructing the library covers a range of molecular weight "Low $\rightarrow$ High". For a library of n-mers, the range of molecular weights Low $\rightarrow$ High is divided into n similar intervals, each one covering a sub-range of molecular weights equal or approximately equal to $(\text{High} - \text{Low})/n$.

Each interval of molecular weight is assigned to a position on the scaffold. The position can increase with increasing the molecular weight (i.e. the functional hydroxyls belonging to the interval with lowest mass are used to functionalize the first position, the functional hydroxyls belonging to the next higher mass interval are used to functionalize the second position, etc ...), or decrease with increasing the molecular weight, or be arbitrarily assigned.

After cleavage, the solution is analyzed by mass spectrometry to result in a mass spectrum exemplified by the following:



This model spectrum indicates that:

- 1) the functional hydroxydes belonging to the lowest mass range (group 1) were used to functionalize position 1, and that the the 8th functional hydroxide of that group was coupled to the selected bead;

- 2) the functional hydroxydes belonging to the next higher mass range (group 2) were used to functionalize position 2, and that the the 18th functional hydroxide of that group was coupled to the selected bead;
- 3) the functional hydroxydes belonging to the next higher mass range (group 3) were used to functionalize position 5, and that the the 5th functional hydroxide of that group was coupled to the selected bead.
- 4) the functional hydroxydes belonging to the next hier mass range (group 4) were used to functionalize position 6, and that the the 10th functional hydroxide of that group was coupled to the selected bead.
- 5) the functional hydroxydes belonging to the next higher mass range (group 5) were used to functionalize position 4, and that the the 7th functional hydroxide of that group was coupled to the selected bead.
- 6) the functional hydroxydes belonging to the highest mass range (group 6) were used to functionalize position 3, and that the the 21th functional hydroxide of that group was coupled to the selected bead.

Notably, each group of functional hydroxyls can comprise a different number of items, which are numbered according to their increasing molecular weight. In the example proposed, the 8th hydroxyl of group 1 is almost on the right edge of the mass range (used for position 1). Group 6, used for position 3, has more than 21 hydroxyls, as the 21th is approximately at the middle of the mass range explored by group 6.

The case be considered of a library of hexamers constructed using 100 functional hydroxyls, exploring an overall range of molecular weight Low-High.

Six intervals are assigned, each of extent $(\text{High} - \text{Low}) / 6$. Each group, i.e. each mass interval, may contain a different number of functional hydroxyls. For example, group 1 may have 10, group 2 may have 20, group 3 may have 7, group 4 may have 25, group 5 may have 11, and group 6 may have 27.

As in the model spectrume above, the functional hydroxyls of group 1 are used to functionalized position 1, the functional hydroxyls of group 2 are used to functionalized position position 2, the functional hydroxyls of group 3 are used to functionalized position position 5, the functional hydroxyls of group 4 are used to functionalized position position 6,

the functional hydroxyls of group 5 are used to functionalized position position 4, and the functional hydroxyls of group 6 are used to functionalized position position 3.

After library screening, the beads are sequenced using mass spectrometry data, as illustrated in the model spectrum above.

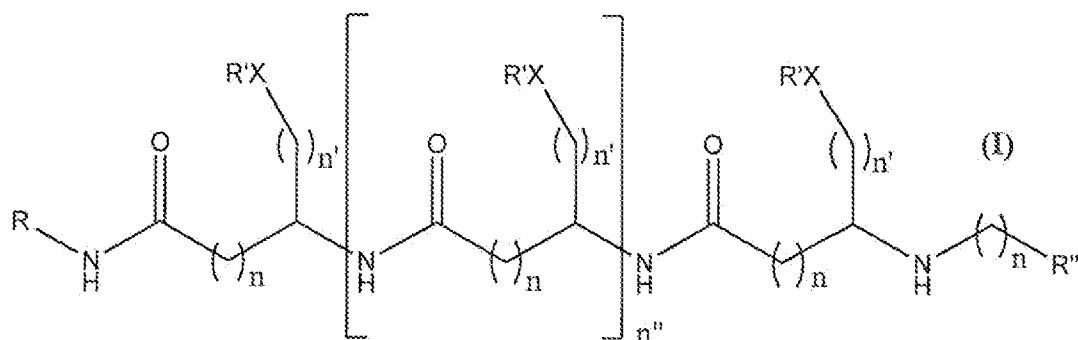
This hence represents an extremely fast method for sequencing the compounds on the selected beads. This strategy is also amenable to the design of high-throughput screening methods, which are sought after in drug research.

When, finally, the selected compound is re-synthesized in bulk, in some instances the use of ester bonds may not be desired. It is hence possible to replace ester with amide bonds (as, for example in the compounds of **Formula Ic** above). The backbone (protecting groups) being the same, the iterative deprotection-coupling procedure is the same, except that compounds of **Formula IIIc** are employed rather than compounds of **Formula IIIb**.

The foregoing is illustrative of the present invention, and is not to be taken as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A method of making a peptide of **Formula I**:



wherein:

R is a solid support;

each R' is an independently selected functional group;

R'' is a functional group;

X is a linking group;

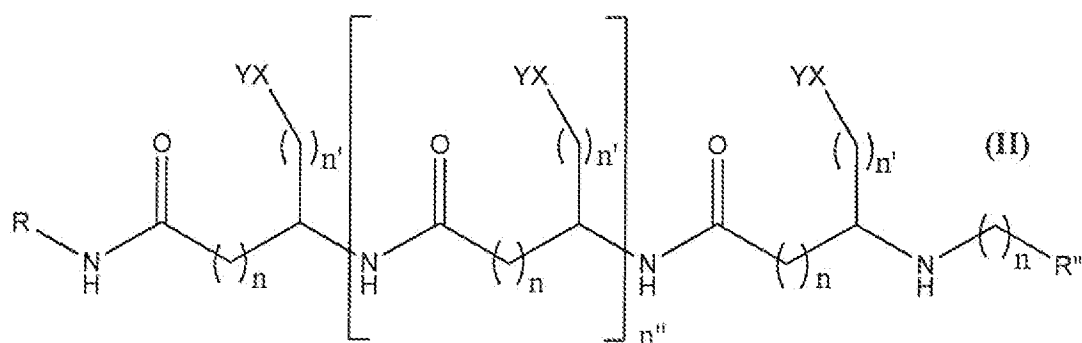
each n is 0, 1 or 2;

each n' is 0 or an integer of from 1 to 5; and

n'' is an integer of from 1 to 20;

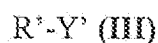
said method comprising the steps of:

(a) removing at least one protecting group from a compound of **Formula II**:



where R, R'', n, n' and n'' are as given above and each Y is an independently selected protecting group to produce a deprotected intermediate; then

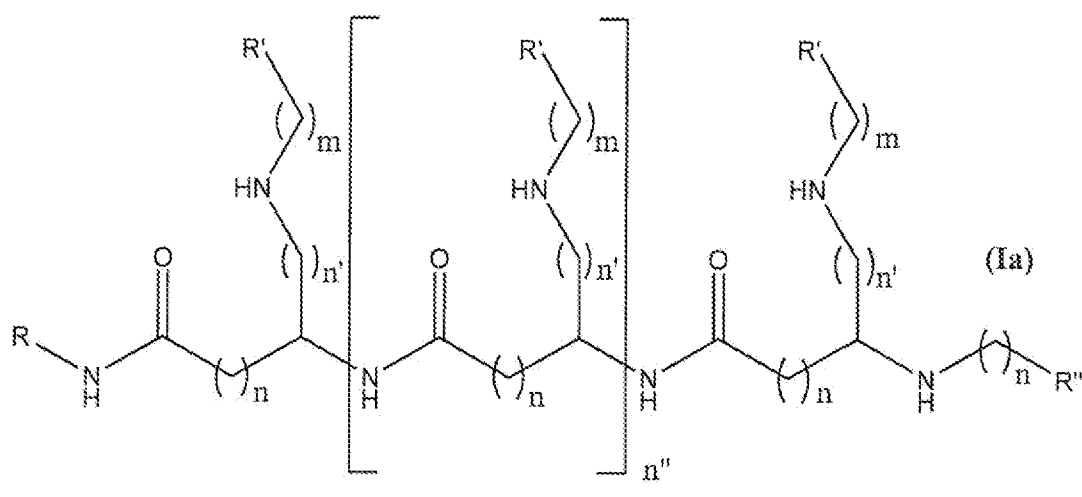
(b) reacting said deprotected intermediate with compound of **Formula III**:



where R' is a functional group as given above and Y' is a reactive or functional group, to replace said at least one protecting group Y removed in step (a) with said functional group R' ; and then

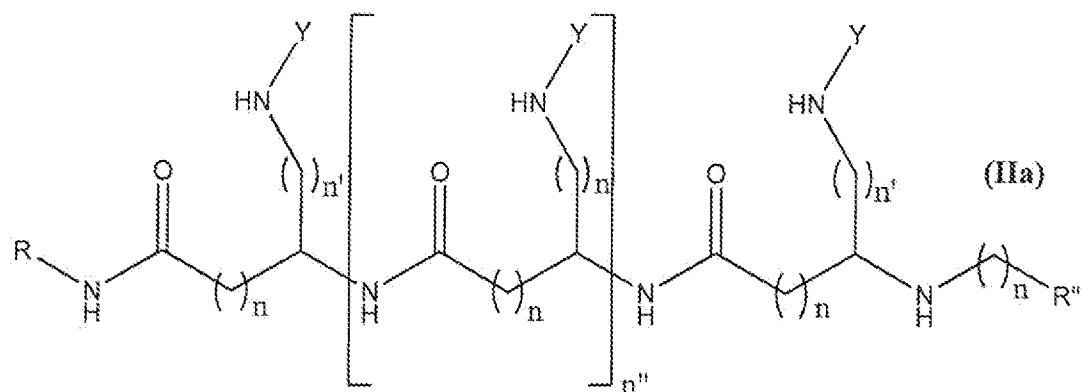
(c) iteratively repeating steps (a) and (b) at least once until all of said protecting groups Y are replaced with said functional groups R' .

2. The method of claim 1, wherein said compound of **Formula I** is a compound of **Formula Ia**:

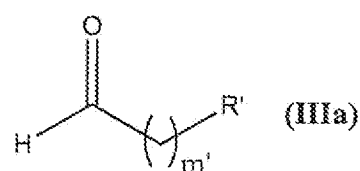


where each m is independently 1, 2 or 3;

said compound of **Formula II** is a compound of **Formula IIa**:



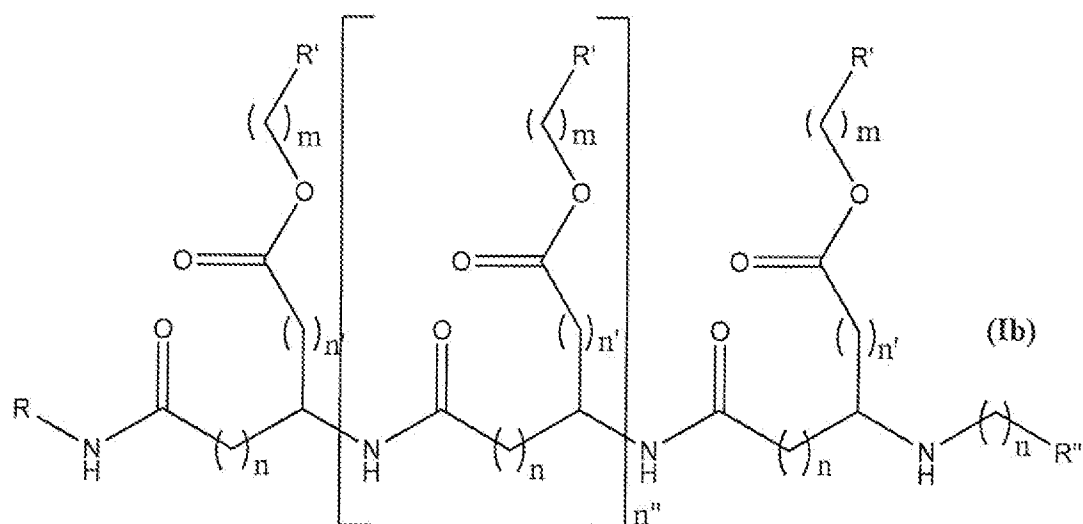
and said compound of **Formula III** is a compound of **Formula IIIa**:



where m' is 0, 1 or 2.

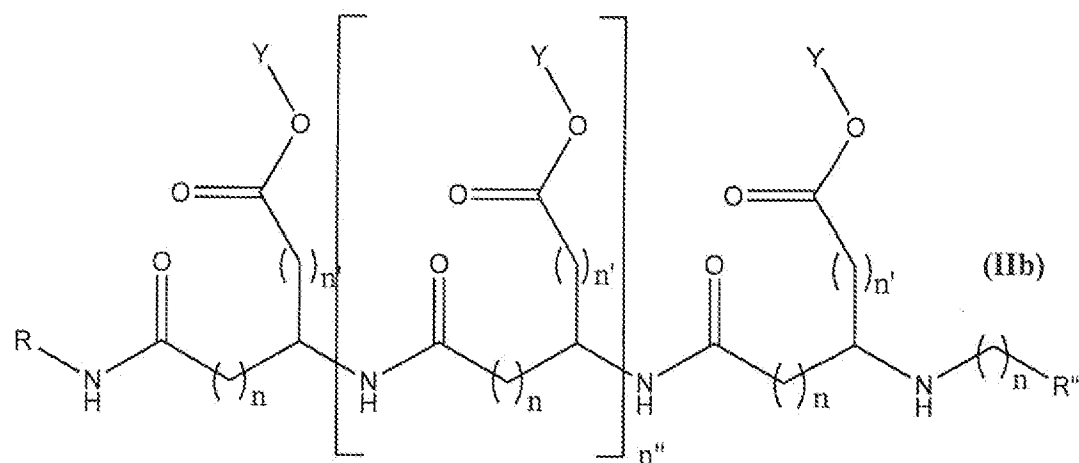
3. The method of claim 2, wherein said reacting step (b) comprises a reductive amination reaction.

4. The method of claim 1, wherein said compound of **Formula I** is a compound of **Formula Ib**:

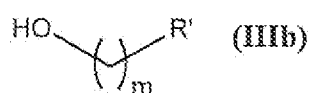


where each m is independently 1, 2 or 3;

said compound of **Formula II** is a compound of **Formula IIb**:



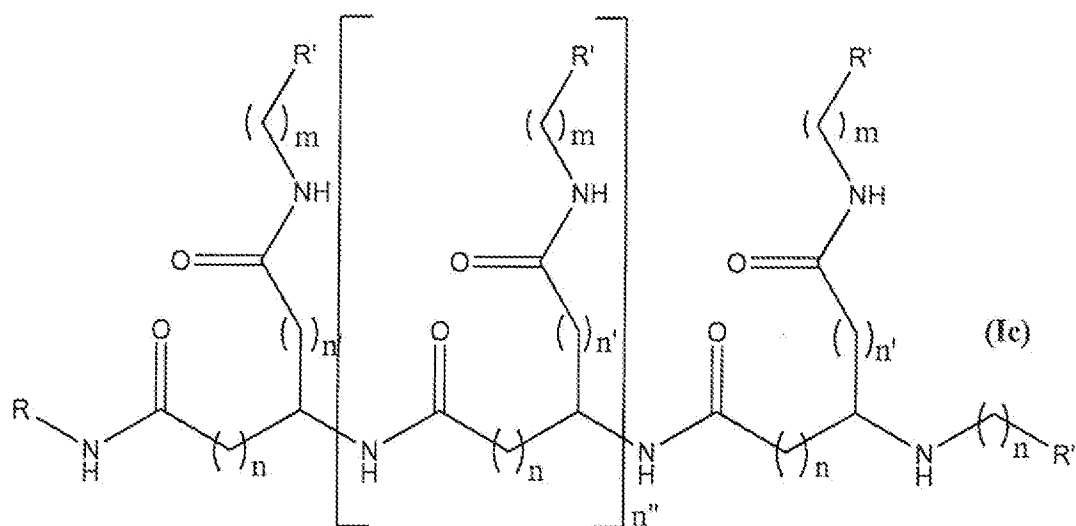
and said compound of **Formula III** is a compound of **Formula IIIb**:



where m is as given above.

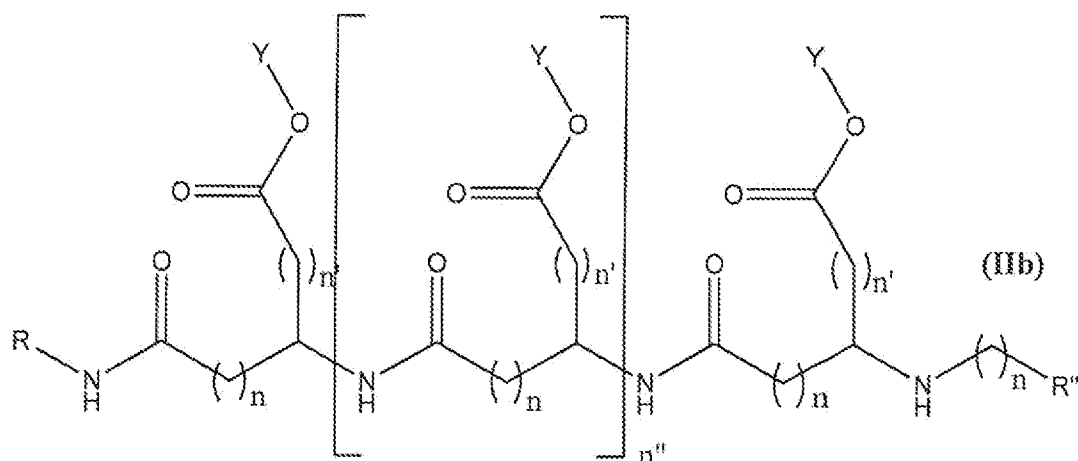
5. The method of claim 4, wherein said reacting step (b) comprises an esterification reaction.

6. The method of claim 1, wherein said compound of **Formula I** is a compound of **Formula Ic**:

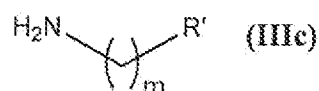


where each m is independently 1, 2 or 3;

said compound of **Formula II** is a compound of **Formula IIb**:



and said compound of **Formula III** is a compound of **Formula IIIc**:



where m is as given above.

7. The method of claim 1, wherein said reacting step (b) comprises an amidation reaction.

8. The method of claim 1 to 7, wherein:

at least three of said functional groups R' are different from one another; and
at least three of said protecting groups Y are different from one another.

9. The method of claim 8, wherein like protecting groups Y removed in each step (b) are replaced by like or different functional groups R' in each immediate subsequent step (c).

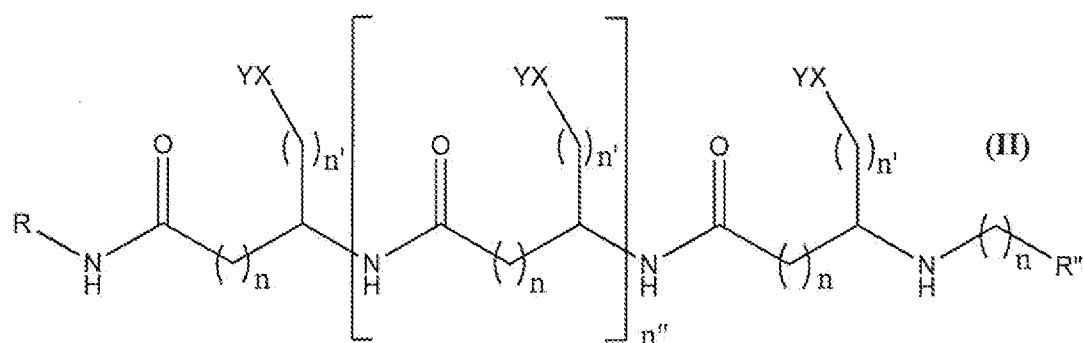
10. The method of claims 1 to 9, wherein said iteratively repeating is carried out at least twice.

11. The method of claims 1 to 10, wherein each of said protecting groups Y are independently selected from the group consisting of alkaline-stable amino protecting groups,

alkaline-labile amino protecting groups, alkaline-stable carboxylic acid protecting groups, and alkaline-labile carboxylic acid protecting groups.

12. The method of claim 1 to 11, wherein each R' is independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloalkylalkenyl, cycloalkylalkynyl, heterocyclo, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, aryl, arylalkyl, arylalkenyl, arylalkynyl, heteroaryl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, alkoxy, halo, mercapto, azido, cyano, formyl, carboxylic acid, hydroxyl, nitro, acyl, aryloxy, alkylthio, amino, alkylamino, arylalkylamino, disubstituted amino, acylamino, acyloxy, ester, thioester, carboxylic thioester, ether, amide, amidine, sulfate, sulfoxyl, sulfonyl, sulfonate, sulfonic acid, sulfonamide, urea, alkoxylacylamino, aminoacyloxy, guanidino, aldehyde, keto, imine, nitrile, phosphate, thiol, epoxide, peroxide, thiocyanate, amidine, oxime, nitrile, and diazo.

13. A compound of **Formula II**:



wherein:

R is a solid support;

each R' is an independently selected functional group;

R'' is a functional group;

each n is 0, 1 or 2;

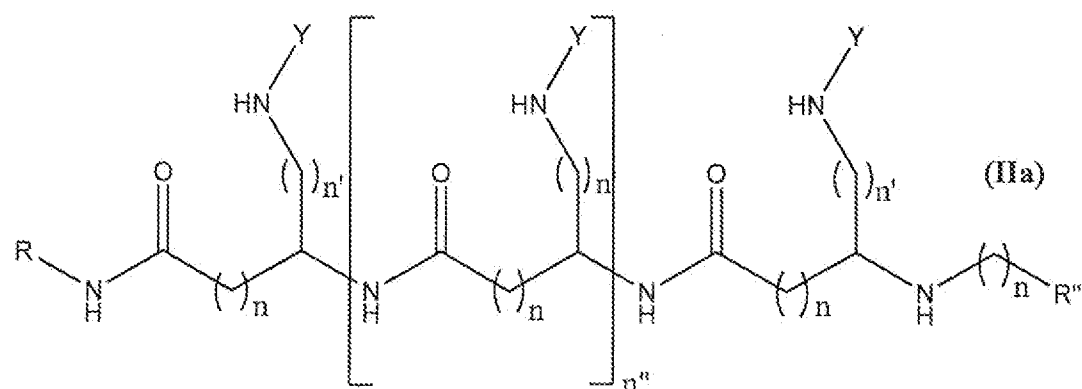
each n' is 0 or an integer of from 1 to 5; and

n'' is an integer of from 1 to 20;

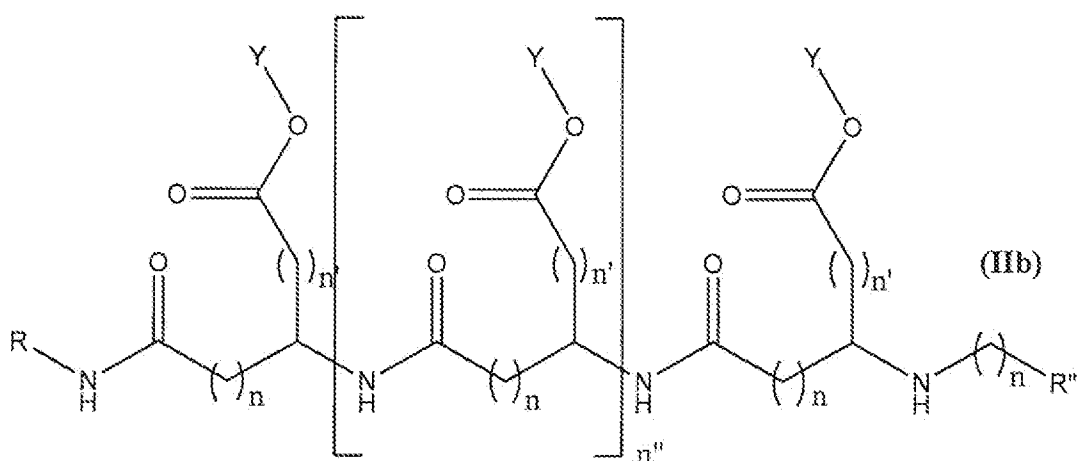
each X is a linking group; and

each Y is an independently selected protecting group.

14. The compound of claim 13, wherein said compound of **Formula II** is a compound of **Formula IIa**:



15. The compound of claim 13, wherein said compound of **Formula II** is a compound of **Formula IIb**:



16. The compound of claim 13 to 15, wherein each X is an amine or ester linking group.

17. The method of claim 13 to 16, wherein at least three of said protecting groups Y are different from one another.

18. The compound of claim 13 to 17, wherein n'' is at least 3.

each R' is an independently selected functional group,

R'' is a functional group;

each n is 0, 1 or 2;

each n' is 0 or an integer of from 1 to 5;

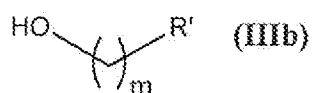
n'' is an integer of from 1 to 20;

each R' is selected from a set of known functional groups of different molecular weights' and;

said peptide of Formula Ib is synthesized as a member of a library with known subsets of functional groups within a known molecular weight interval at known positions in said peptide

said method comprising the steps of:

(a) simultaneously hydrolyzing all of said ester linkages to produce a composition comprising n'' + 2 compounds of **Formula IIIb**:



where m and R' is as given above;

(b) determining the molecular weight of each of said n'' + 2 compounds of **Formula IIIb** in said composition;

(c) determining the identity of each functional group R' from (i) the molecular weight of each of said compounds of Formula IIIa in said composition determined in step (b) and (ii) the set of known functional groups of different molecular weights; and then

(d) determining the position of each of said functional groups R' on said composition from (i) either the identity or the molecular weight of said functional group R', and (ii) the known position in said peptide for the known subset of functional groups of known molecular weights to which said functional group belongs.

23. The method of claim 22, wherein said determining step (b) is carried out by mass spectrometry.

24. The method of claim 22 to 23, wherein:

at least three of said functional groups R' are different from one another; and

at least three of said protecting groups Y are different from one another.

25. The method of claim 22 to 24, wherein each R' is independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, cycloalkylalkenyl, cycloalkylalkynyl, heterocyclo, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, aryl, arylalkyl, arylalkenyl, arylalkynyl, heteroaryl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, alkoxy, halo, mercapto, azido, cyano, formyl, carboxylic acid, hydroxyl, nitro, acyl, aryloxy, alkylthio, amino, alkylamino, arylalkylamino, disubstituted amino, acylamino, acyloxy, ester, thioester, carboxylic thioester, ether, amide, amidine, sulfate, sulfoxyl, sulfonyl, sulfonate, sulfonic acid, sulfonamide, urea, alkoxylacylamino, aminoacyloxy, guanidino, aldehyde, keto, imine, nitrile, phosphate, thiol, epoxide, peroxide, thiocyanate, amidine, oxime, nitrile, and diazo.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/73805

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C40B 40/10, 50/04, 50/18 (2014.01)

USPC - 530/333, 334, 335; 506/18, 23

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C40B 40/10, 50/00, 50/04, 50/18 (2014.01)

USPC: 530/333, 334, 335; 506/18, 23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; PubMed; IP.com; North Carolina State University, Menegatti, Carbonell, Naik, peptide, solid phase synthesis, functional group, amide, esterification, reductive amination, protein sequencing, hydrolysis, polyether, allyl, benzyl, propargyl

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	GERONA-NAVARRO, G et al. Solid-Phase Synthesis of a Library of Amphipatic Hydantoins. Discovery of New Hits for TRPV1 Blockade. ACS Comb Sci., Vol. 13, No. 5, September 12, 2011, pp. 458-465, National Institutes of Health Public Access [online], [retrieved on 2014-02-28]. Retrieved from the Internet <URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3296451/pdf/nihms359978.pdf > <DOI: 10.1021/co1000986>; page 15, scheme 1, peptides 2, 3, 4-(G1-2), 6-(G1-2, A1-14), legend for scheme 1	1, 13-14, 16/13-14 --- 2-3, 8/1-3, 9/8/1-3
X --- Y --- A	EP 0 518 295 A2 (HUDSON, D et al.) December 16, 1992; page 13, lines 15-25; page 14, lines 12-20	1, 6-7, 13, 15, 16/13, 16/15 --- 4-5 --- 8/4-7, 9/8/4-7, 22-23, 24/22-23
Y	RAINDLOVA, V et al. Synthesis of Aldehyde-Linked Nucleotides and DNA and Their Bioconjugations with Lysine and Peptides through Reductive Amination. Chemistry - A European Journal, Vol. 18, Iss. 13, March 26, 2012, pp. 4080-4087 [online], [retrieved on 2014-02-28]. Retrieved from the Internet <URL: http://onlinelibrary.wiley.com/doi/10.1002/chem.201103270/abstract?deniedAccessCustomisedMessage=&userIsAuthenticated=false > < DOI: 10.1002/chem.201103270>; abstract	2-3, 8/2-3, 9/8/2-3

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 March 2014 (06.03.2014)

Date of mailing of the international search report

21 MAR 2014

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Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/73805

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROBERTS, MJ et al. Chemistry for Peptide and Protein PEGylation. Advanced Drug Delivery Reviews, Vol. 54, 2002, pp. 459-476, Elsevier [online], [retrieved on 2014-02-28]. Retrieved from the Internet <URL: http://www.laysanbio.com/clientuploads/Chemistry-20for-20peptide-20and-20protein-20PEGylation.pdf >; page 461, column 2, paragraph 4	4-5
Y --- A	US 2004/0086949 A1 (HOLM, A et al.) May 6, 2004; paragraphs [0085], [0091], [0097], [0119]	8/1-3, 9/8/1-3 ---
A	US 7,521,529 B2 (HILLMAN, JD et al.) April 21, 2009; column 7, lines 1-14	8/4-7, 9/8/4-7
A	SPIVEY, AC et al. Synthetic methods Part (iv) Protecting groups. Annu. Rep. Prog. Chem., Sect. B, Vol. 96, 2000, pp. 65-83 [online], [retrieved on 2014-02-28]. Retrieved from the Internet <URL: https://workspace.imperial.ac.uk/spiveygroup/Public/publicationpdfs/annurepprogchemsectb20009665.pdf > < DOI: 10.1039/b003402i>; page 73, paragraph 1	8/4-7, 9/8/4-7
A	US 5,470,753 A (SEPETOV, N et al.) November 28, 1995; abstract	22-23, 24/22-23
A	US 2010/0240139 A1 (OH, HB et al.) September 23, 2010; abstract	22-23, 24/22-23
A	US 2010/0237238 A1 (FRANZEN, J) September 23, 2010; paragraph [0001]	22-23, 24/22-23
A	US 2011/0071040 A1 (SHIN, SK et al.) March 24, 2011; paragraph [0001], [0035]	22-23, 24/22-23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/73805

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 10-12, 17-21 and 25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.